REMARKS

Applicant's attorney wishes to thank Examiner Fredman for the courtesies extended during the interview of December 4, 2002.

Claims 19-36 currently appear in this application. The Office Action of August 14, 2002, has been carefully studied. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicants respectfully request favorable reconsideration, entry of the present amendment, and formal allowance of the claims, as the amendments to the claims are made solely to clarify a point that had been considered previously, namely, that the probe be stably incorporated at the lipid surface, or, in other words, that the probe be retained at the membrane surface. Therefore, no new issues are raised, and entry of the present amendment is respectfully requested.

The Present Invention

The present invention provides a method for determining **binding of a species at a surface** (in which the surface is a well characterized surface having a local environment at a given and know pH or surface potential), wherein the binding is effective to alter the pH or potential at the surface. This method comprises

incorporating **at the surface** a probe (which comprises a pH- or potential-sensitive fluorophore attached to a steroid, to a head group of a sphingolipid, or to a head group of a lipid having at least two chains, each chain comprising at least 14 carbon atoms in length), **wherein the incorporation of the probe at the surface is not altered upon binding or dissociation of the species at the surface**, and observing a change in a fluorescent property of the fluorophore **which is retained at the surface** upon binding or dissociation of the species at the surface.

Claims 19 and 34 have been amended to clarify the invention for which protection is sought, namely, a method for determining binding species of a polymer surface in which a pH- or potential-sensitive fluorophore is incorporated at the polymer surface. In the present invention, "stably" means that incorporating the fluorophore at the surface is substantially not altered by the binding or dissociation of the species at the surface. Support for this amendment can be found in the specification as filed at page 9, line 36 through page 10, line 14.

Claims 19-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zuidam et al. in view of Gee et al. The Examiner's position is that Zuidam et

al. teach a method for determining binding of a nucleic acid species at a lipid surface, and that Gee et al. teach probes that possess a lipophilic moiety and therefore can be stably incorporated into lipid assemblies for use as probes for membrane structures, etc.

This rejection is respectfully traversed. The claims have been amended to emphasize that the probe in the instant invention remains at the surface during the entire analytical experiment. Unlike in Zuidam et al., in which the probe is detached once the analysis is in process in the biologically relevant media (see, for example, Subsection 3.1 in Zuidam et al.), the association or dissociation of the analyte to be detected according to the invention does not influence the incorporation of the probe at the surface, as described in the specification of the instant application, particularly at page 9, line 36 through page 10, line 14.

Zuidam et al., as the Examiner admits, presents a study which is aimed at characterizing membranes. The variable that changes in the assay of Zuidam et al. is the type of lipids comprising the surface with which a DNA is complexed. According to Zuidam et al., the DNA is a known and well characterized parameter which is used in order to determine the behavior of differently composed

lipidic surfaces. However, in the present invention, the variable to be characterized is the analyte in a sample. The present invention is aimed at determining the dissociation or binding properties of an unknown analyte to a well-defined and characterized surface, using a well defined and characterized probe.

During the interview of December 4, 2002, the Examiner suggested submitting a declaration showing that Zuidam et al. do not meet the limitation that the incorporation of the probe is not substantially altered upon binding or dissociation of the species at the surface. However, it is believed that the data in the specification as filed are sufficient to demonstrate that Zuidam et al. do not meet this limitation. The improvement of the present invention over Zuidam et al. is in modification of the probe, which modification prevents alteration of the probe upon binding or dissociation of the species.

The present specification as filed demonstrates that the probe is stably incorporated at the surface of the liposomes, particularly in Table 1 on page 10. Table 1 shows that when using a fluorophore modified according to the present invention (in the specific example, 4-heptyl 7-hydroxycoumarin modified with a dialkyl, diacyl or dialkenyl phosphatidyl ethanolamine (HC-PE), there is

substantially no dissociation of the probe from the surface (F/F0 substantially equals 1), while when using an unmodified fluorophore (HC), such as that employed by Zuidam (see Results on page 117 of Zuidam), the incorporation of the probe at the surface is unstable (F/F0 is 0.65).

This clearly demonstrates that the modification of the probe as claimed herein makes it possible to incorporate the probe at the surface of the species because the incorporation of the probe at the surface of the species is substantially not altered upon binding or dissociation of the species at the surface.

Although Gee et al. provide lipophilic derivatives of dye compounds, Gee et al. specifically state that these lipophilic derivatives will incorporate **into** lipid assemblies, *e.g.*, for use as probes for membrane structure or **for incorporation into liposomes, lipoproteins, films, plastics, lipophilic microspheres or similar materials** (column 23, lines 23-35). Gee et al. also state that embodiments of the invention that have lipophilic moieties have enhanced penetration of the substrate into live cells and improved retention of the fluorescent product in the cells.

One skilled in the art reading Zuidam et al. and Gee et al. would see that Zuidam et al. are concerned

with characterizing membranes, and that the probes of Gee et al. can be used for this purpose. However, there is no teaching or suggestion in Gee et al. of probes which are retained at a surface which makes it possible to determining binding of species at the surface. In fact, Gee et al. **teach away** from the method of the present invention, since Gee et al. suggest that fluorophores modified according to Gee et al. (*e.g.*, with a lipophilic moiety) would penetrate **into** the surface. Because the Gee et al. probes penetrate into the surface, they would not act as **surface** probes, as in the present invention.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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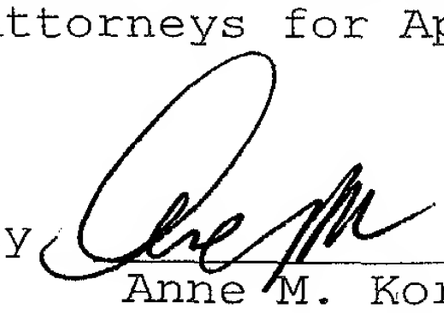
By 
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19. (Amended) A method for determining binding of a species at a surface having a local environment at a given pH or surface potential, wherein said binding is effective to alter said pH or potential, the method comprising:

~~stably~~ incorporating at said surface a probe which comprises a pH- or potential-sensitive fluorophore attached to a steroid, to a head group of a sphingolipid or to a head group of a lipid having at least two chains, each chain comprising at least 14 carbon atoms in length, and wherein each independently said chain is selected from the group consisting of acyl, alkyl or alkenyl, **wherein incorporation of the probe at the surface is substantially not altered upon binding or dissociation of the species at the surface** and

observing a change in a fluorescent property of said fluorophore **retained at the surface** upon binding or dissociation of said species at said surface.

34. (Amended) A method for determining binding of a species at a polymer surface having a local environment at a given pH or surface potential, wherein said binding is effective to alter said pH or potential, the method comprising:

~~stably~~ incorporating at said polymer surface a pH- or potential-sensitive fluorophore **wherein incorporating the fluorophore at the surface is substantially not altered upon binding or dissociation of the species at the surface**, and

observing a change in a fluorescent property of said fluorophore **retained at the surface** upon binding or dissociation of said species at said surface.